PEGYLATION OF POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is filed as a continuation-in-part of United States Patent Application Serial No. 07/822,296, filed January 17, 1992 entitled Method for Treating Tumor Necrosis Factor Mediated Diseases, United States Patent Application Serial No. 07/669,862, filed March 15, 1991 entitled Site Specific Pegylation of Polypeptides, United States Patent Application Serial No. 07/506,522, filed April 6, 1990 entitled Interleukin-1 Inhibitors, and United States Patent Application Serial No. 07/555,274 filed July 19, 1990 entitled Tumor Necrosis Factor (TNF) Inhibitor and Method for Obtaining Same.

FIELD OF THE INVENTION

This invention relates to polypeptides that have been covalently bonded to long chain polymers such as methoxy polyethylene glycol. This invention also describes methods and reagents for the reaction of activated polymer molecules with various biologically-important polypeptides.

BACKGROUND OF THE INVENTION

Many proteins that have been identified and isolated from human and animal sources have been found to show promising medicinal or therapeutic potential. Great strides have been made in the methods for identifying and characterizing such proteins, in addition to methods for producing such proteins in relatively pure forms and relatively large quantities. As the development process advances in relation to the utilization of such potentially valuable materials, many obstacles have arisen in formulating these compounds for use in clinical models.

include any single or combination of consensus repeat sequences of CR1), PDGF receptor, IL-2, MCSF receptor, EGF receptor, IL-5 receptor, IL-3 receptor, GMCSF receptor, T-cell receptor, HLA-I, HLA-II, NGF receptor, IgG (V_K, V_l), CD40, CD27, IL-6 receptor, Integrins CR3, VLA_4, ICAM, and VCAM, CR2, GMP140 Lec domain, Laminin binding protein, Laminin fragments, Mannose binding protein, exon 6 peptide of PDGF, and proteases (with 2 catalytic domains or a target domain and a catalytic domain). All references to receptors includes all forms of the receptor whenever more than a single form exists. In the preferred embodiments, the groups R_1 and R_2 are selected from the group consisting of IL-1 receptor antagonist, 30kDa TNF inhibitor, CR1, and IL-2 receptor (both the α and β chains).

In a preferred embodiment, the non-peptidic polymeric spacer X may be further defined as follows: X = $-Y_1-(Z)_n-Y_2-$, wherein Y_1 and Y_2 represent the residue of activating groups that react with R_1 and R_2 to link the spacer to the groups R_1 and R_2, and $(Z)_n$ represents the base polymeric group. According to the present invention n is greater than 6 and preferably is greater than 10.

Non-peptidic is defined as a polymeric group that is substantially not peptidic in nature. The inclusion of less than 50% by weight of α-amino acid residue as part of Y_1, Y_2 and Z would be considered substantially non-peptidic in nature and would be considered non-peptidic. In the preferred embodiment, the non-peptidic spacer X is non-immunogenic, and biologically inert and hydrophilic. In addition, the preferred linkers are capable of conveying desirable properties to the biologically active polypeptidic groups -- such as reduced immunogencity, increased solubility, or reduced clearance rate from the body -- without significantly reducing the affinity of a given R_1 or R_2 group to its ligand. In the most preferred

embodiments, the compound R_1-X-R_2 (wherein R_1=R_2 and R_1 and R_2 are binding groups) has an affinity for its ligand that exceeds the affinity that the non-derivitized binding group has to the ligand. For example, substantially purified c105 30kDa TNF inhibitor PEG_{3400}db has an inhibitor activity for TNF that is greater than 20 times the inhibitor activity that c105 30kDa TNF inhibitor has for TNF.

The activating groups Y_1 and Y_2 that are part of the polymeric spacer X may be comprised of any of the activating groups as discussed above, including the maleimide group, sulfhydryl group, thiol, triflate, tresylate, aziridine, oxirane, and 5-pyridyl. The preferred activating groups are maleimides.

The polymeric group $(Z)_n$ is preferably selected from the group consisting of polyethylene glycol, polypropylene glycol, polyoxyethylated glycerol, dextran, poly β-amino acids, colonic acids or other carbohydrate polymers and polymers of biotin derivatives. In the preferred embodiments, the polymeric group is polyethylene glycol. Any non-peptidic polymeric group that would serve the functions as described herein would also be included within the scope of this invention.

One of the advantages of the present invention is the ability to vary the distance between the groups R_1 and R_2 by varying the length of the polymeric group linking the two binding groups. Although not limited by theory, it is proposed that the increase in biological activity seen for the multimeric compounds of this invention may be attributed to the multimeric nature of the cell receptors and ligands <u>in vivo</u>. For this reason, the optimal distance between the units R_1 and R_2 (which would be generally directly proportional to the length of the polymeric unit $(Z)_n$) may be easily determined by one skilled in the art by varying the size of the spacer X.

In one embodiment of the present invention, the